The Structure of Arachidonic and Linoleic Acids

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Early in 1940 Shinowara & Brown described the results of the ozonolysis of methyl arachidonate and of its oxidation by $KMnO_4$ in acetone solution. As a result of their experiments they formulated arachidonic acid as $\Delta^{6.10.14.18}$-eicosatetrenoic acid (I):

$$CH_3.CH{:}CH.CH_2.CH_2.CH{:}CH.CH_2.CH_2.CH{:}CH.$$
$$CH_2.CH_2.CH{:}CH.CH_2.CH_2.CH_2.CH_2.CO_2H \quad (I)$$

They emphasized that this structure was not fully confirmed and must be regarded only as a tentative suggestion. Before the appearance of this paper, Dolby, Nunn & Smedley-MacLean had for some time been engaged in elucidating the structure of this acid, using the method of alkaline permanganate oxidation and as their results were entirely at variance with those obtained by Shinowara & Brown, they published a preliminary account [1940] indicating that arachidonic acid was $\Delta^{5.8.11.14}$-eicosatetrenoic acid (II):

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH{:}CH.CH_2.CH{:}CH.$$
$$CH_2.CH{:}CH.CH_2.CH{:}CH.CH_2.CH_2.CH_2.CO_2H \quad (II)$$

The evidence for this structure was that glutaric and succinic acids were the only dibasic acids isolated; the amount of glutaric acid obtained was very small but it gave no depression in melting-point when mixed with the pure acid. From the other end of the molecule a fraction of volatile fatty acids was obtained which behaved like a mixture of valeric and caproic acids. The melting-point of the *p*-bromophenacyl caproate separated by fractional crystallization was not raised to that of the pure compound, and it was considered desirable at some future time to confirm this result. The authors felt justified, however, in proposing the formula (II) for arachidonic acid.

From the biological point of view, the structure of arachidonic acid is of considerable importance. According to the results of Smedley-MacLean & Hume [1941] the rat synthesizes clupanodonic acid (docosapentenoic acid) only if linseed oil acids are supplied, and it is considered probable that these acids are also the precursors of arachidonic acid in the body. If the formula of Dolby *et al.* were correct, the 11 terminal carbon atoms of arachidonic and linoleic acids would be identically linked and the biological synthesis would involve the addition of 2 carbon atoms at the carboxyl end of the molecule and the introduction of double bonds at the 5.6 and 8.9 positions of the C_{20} chain or in the 3.4 and 6.7 positions of the C_{18} chain. On the other hand, the tentative formula suggested by Shinowara & Brown bore no resemblance to that of linoleic acid and did not contain the structure

$$—CH{:}CH.CH_2.CH{:}CH—$$

characteristic of the linseed acids. A comparison of the ozonolysis products of methyl arachidonate and ethyl linoleate was therefore undertaken by the present authors, together with an acetone-permanganate oxidation of the former, and the investigation had been almost completed when a communication from Mowry, Brode & Brown [1942] appeared in which the formula (I) tentatively suggested by Shinowara & Brown was withdrawn and that proposed by Dolby *et al.* (II) entirely confirmed. The later work of the American authors had been carried out on a large scale, using 197 g. of arachidonate in place of 11 g. employed in our experiments, enabling them to isolate relatively large quantities of purified oxidation products.

An eicosatetrenoic acid has been isolated by Toyama & Tsuchiya [1935] from fish oil which gave on oxidation only acids containing 4 carbon atoms. They therefore considered it to be $\Delta^{4.8.12.16}$-eicosatetrenoic acid which contains 4 of the groupings

$$—CH{:}CH.CH_2.CH_2—$$

and in which the characteristic linseed acid structure is absent. This acid has been designated arachidonic acid, but it seems certain that it is not identical with the arachidonic acid prepared from ox suprarenal fat, and it is unfortunate that the same name should be employed. We suggest that the name arachidonic acid be reserved for that originally isolated by Hartley [1909] from pig's liver, to which the constitution $\Delta^{5.8.11.14}$-eicosatetrenoic acid is assigned, and that the name should not be used to describe the acid obtained from fish oil.

A survey of the literature showed that apparently the only work on the ozonolysis of linoleic acid was by Takahashi [1921] whose results cannot be reconciled with the commonly accepted formula. This provided an additional reason for completing the present work. Takahashi ozonized the acid prepared from soya-bean and rice-bean oils; among the products he identified normal butyric acid and the corresponding aldehyde, glutaric acid and its dialdehyde, azelaic acid and its half aldehyde. He therefore represented the double bonds as occurring

in the 9:10 and 14:15 positions (III) instead of in the 9:10 and 12:13 positions (IV) as has been generally accepted:

$CH_3CH_2.CH_2.CH{:}CH.CH_2.CH_2.CH_2.CH{:}CH.CH_2.$
$CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CO_2H$ (III)

$CH_3.CH_2.CH_2.CH_2.CH_2.CH{:}CH.CH_2.CH{:}CH.CH_2.$
$CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CO_2H$ (IV)

Takahashi stated that the tetrabromo- and tetrahydroxy-stearic acids obtained from his linoleic acid were identical with those prepared from the linoleic acid of poppyseed oil. Haworth [1929] prepared linoleic acid from both soya bean and poppyseed oils, and obtained products by oxidation with acetone-permanganate, and with alkaline permanganate, which were in agreement with formula (IV). Birosel [1937] found the linoleic acid prepared from soya bean oil identical with that from cottonseed oil; both gave the normal tetrabromo- and tetrahydroxy-stearic acids. Ethyl linoleate has now been prepared from linoleic acid tetrabromide (M.P. 114–115°), obtained from maize oil, and on ozonolysis it gave products in agreement with formula (IV) ($\Delta^{9.12}$-octadecadienoic acid). We therefore do not confirm the results of Takahashi.

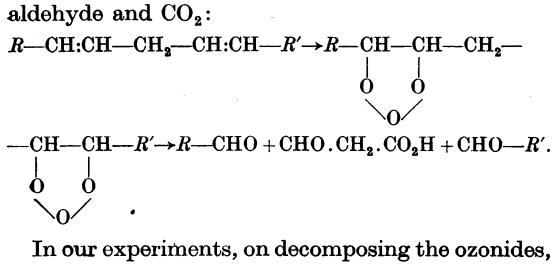
Erdmann, Bedford & Raspe [1909] have ozonized linolenic acid (V) and its esters:

$CH_3CH_2.CH{:}CH.CH_2.CH{:}CH.CH_2.CH{:}CH.$
$CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CO_2H$ (V)

They adopted the view of Harries that the ozonide decomposes giving an atom of nascent oxygen for each ozonide group, and that this oxygen may react with H_2O forming H_2O_2 or may oxidize the aldehydes to acids; under these conditions the lower aliphatic aldehydes are those most easily oxidized. Further oxygen is available because linolenic acid and its ethyl ester, when ozonized in chloroform solution, yield the ozonide peroxide; the increase in weight noted indicates this to be the case also for ethyl linoleate. The ozonide of methyl arachidonate was decomposed without removal of solvent but it is likely that it also is an ozonide-peroxide. Erdmann *et al.* [1909] found that the grouping

—CH:CH.CH_2.CH:CH—

yielded an ozonide which, with water, gave formylacetic acid, the latter decomposed at 60° to acetaldehyde and CO_2:



In our experiments, on decomposing the ozonides, acetaldehyde was trapped in 2:4-dinitrophenylhydrazine solution and CO_2 in NaOH solution.

Methyl arachidonate was prepared from arachidonic acid octabromide obtained from ox suprarenal fat kindly supplied to us by Messrs Allen and Hanburys Ltd. It had the characteristics recorded in the experimental portion. The observed molecular refractivity was 100·4 units, the calculated value being 99·05. It is considered from this result that in pure methyl arachidonate the double bonds are not conjugated, and that the slight exaltation shown is to be ascribed to the incidence of rearrangement of double bonds into conjugated positions in a fraction of the ester during distillation; this is regarded by Farmer & van den Heuvel [1938] as unavoidable except in molecular stills. The exaltation may be due in part to anomalous dispersion, since the refractive index for λ_{5461} was found to be lower than that for λ_{5893}. The products isolated were identified by the melting-point and by the mixed melting-point with an authentic specimen, the dibasic acids directly, the monobasic acids after conversion into *p*-bromophenacyl esters, and the aldehydes after conversion into 2:4-dinitrophenylhydrazones. The amounts of the products were estimated as far as possible, and where the yields are considerable, though none are nearly quantitative, they are recorded in Table 1; in all three experiments the only volatile acids found were caproic and acetic, and on the assumption that only these were present the quantities given in Table 1 were calculated from the weights and equivalent weights of the various sodium salts.

Table 1. *Ozonolysis of methyl arachidonate*

	Methyl arachidonate	CO_2	Acetaldehyde 2:4-dinitrophenylhydrazone	Na caproate	Na acetate
Wt. (g.)	5·74	0·966	1·976	0·686	2·046
Moles ($\times 10^{-3}$)	18·0	22·0	8·8	5·0	25·0
Ratio	1·0	1·2	0·5	0·3	1·4

Glutaric acid was identified and the presence of caproic aldehyde indicated. The figure given for sodium acetate must be regarded as approximate.

Oxidation of methyl arachidonate by acetone-$KMnO_4$

	Methyl arachidonate 5·0 g. less 1·0 g. recovered	CO_2	Acetic acid	Caproic acid
Wt. (g.)	4·0	2·38	0·86	0·82
Moles ($\times 10^{-3}$)	12·6	54·1	14·3	7·1
Ratio	1·0	4·3	1·1	0·6

Oxalic acid was identified and succinic acid and caproic acid were indicated. A hydrocarbon identified as n-$C_{27}H_{56}$, and polymerized material (0·63 g.) were also isolated.

Ozonolysis of ethyl linoleate

	Ethyl linoleate	CO_2	Na caproate	Azelaic acid
Wt. (g.)	3·37	0·259	0·848	0·753
Moles ($\times 10^{-3}$)	10·9	5·9	6·1	4·0
Ratio	1·0	0·5	0·6	0·4

Acetaldehyde 2:4-dinitrophenylhydrazone was identified.

The caproic acid found in the ozonolysis of methyl arachidonate indicates that the molecule contains the fragment

$$CH_3.CH_2.CH_2.CH_2.CH_2.CH:$$

The non-volatile fraction was easily oxidized by permanganate giving a product containing an ester group and a carboxyl group, and saponification of this material gave glutaric acid as the only product detected. This sequence of reactions is consistent with the conversion of (impure) glutaric half-aldehyde methyl ester into methyl hydrogen glutarate and then to glutaric acid indicating the terminal portion of the molecule to be

$$:CH.CH_2.CH_2.CH_2.COOMe$$

The remaining 9 carbon atoms yielded 1·9 moles of acetyl radical (as acid and aldehyde) which certainly indicated 3 rather than 2 moles. The yield of CO_2 is low, but comparison with the ethyl linoleate experiment points to the presence of 3 moles of CO_2. Having regard to the results of Erdmann *et al.* [1909] three

$$:CH.CH_2.CH:$$

groups are present and must be adjacent. From these results it is concluded that arachidonic acid has the formula (II).

Farmer & van den Heuvel [1938] oxidized with acetone-permanganate a methyl docosahexenoate containing several

$$:CH.CH_2.CH:$$

groups and found that the ethylenic carbons were oxidized to carboxyl so that these groups gave (presumably via malonic acid) acetic acid. Haworth [1929] oxidized linoleic acid with this reagent, and found a similar rupture of the ethylenic groups, but here the malonic acid, traces of which were detected, gave oxalic acid. We have oxidized methyl arachidonate under conditions resembling those of Farmer & van den Heuvel [1938]. The reaction did not proceed smoothly; 20 % of unoxidized ester was recovered after 37 hr., a considerable amount of polymerized material was formed, and a small quantity of a hydrocarbon, identified by melting-point and analysis as n-$C_{27}H_{56}$, was isolated. The last must have been formed under the somewhat strenuous and prolonged reaction conditions; it was not encountered during ozonolysis. It is considered that the methyl arachidonate was slowly oxidized at the double bonds, giving caproic acid, malonic acid (which mainly decarboxylated to give acetic acid but which was also oxidized to oxalic acid) and glutaric acid, which was further oxidized to succinic acid. (The reaction mixture becomes strongly alkaline and apparently hydrolyses the ester group.)

Ozonolysis of the ethyl linoleate yielded caproic acid, indicating the grouping

$$CH_3CH_2.CH_2.CH_2.CH_2.CH:$$

The sequence azelaic half-aldehyde ethyl ester, ethyl hydrogen azelate, azelaic acid could be followed fairly precisely giving the terminal part of the molecule as

$$:CH.(CH_2)_7.COOOEt$$

The acetaldehyde and CO_2 indicate the remaining 3 carbon atoms to have been

$$:CH.CH_2.CH:$$

The commonly accepted formula (IV) for linoleic acid is therefore confirmed.

EXPERIMENTAL

Melting-points and boiling-points are corrected.

Ozonolysis of methyl arachidonate. Methyl arachidonate was prepared by reduction of arachidonic acid octabromide (60 g.) with zinc (60 g.) in methyl alcohol (600 ml.) containing HCl and subsequent esterification according to Ault & Brown [1934]. It (6·25 g.) had b.p. 177–178°/0·35 mm., i.v. Hübl = 310 (calculated for $C_{19}H_{31}COOCH_3$, 319) and $n_D^{24°}$ 1·4828, $n_{5461}^{24°}$ 1·4811, $\delta_{4°}^{20°}$ 0·905, whence R_D obs. = 100·4; R_D calc. 99·0 [Eisenlohr, 1911] or 99·05 [Swietoslowski, 1920].

The ester (5·86 g.) was dissolved in chloroform (40 ml.), cooled in ice and ozonized by passing a current of 8 % ozone through the solution; portions of the solution were withdrawn for test with Br in CCl_4, until (after 22 hr.) no unsaturation remained. Water (45 ml.) was added to the ozonide solution, which was cloudy and smelt aldehydic. The whole was slowly heated to 98° in a distilling flask connected by ground joints to a condenser and a receiver, and thence to a wash bottle containing 600 ml. of saturated solution of 2:4-dinitrophenylhydrazine in 2N HCl, and a long wash tube containing N NaOH (175 ml.) in a column 26 cm. high; CO_2-free air was aspirated through the apparatus. The hydrazine solution was then removed and replaced by fresh reagent and steam passed, aspiration being continued. In this way CO_2, acetaldehyde, non-volatile, and volatile fractions were separated.

The ice-cooled NaOH solution was titrated with N H_2SO_4 using phenolphthalein and then methyl orange, and 0·966 g. CO_2 were found. A slight excess of H_2SO_4 was added and 87 % of the solution was distilled off; the distillate contained volatile acid equivalent to only 2 % of the found CO_2.

The 2:4-dinitrophenylhydrazine solution yielded crude acetaldehyde 2:4-dinitrophenylhydrazone (1·875 g. m.p. of the two ppts. 142–144° and 149–159·5°) from which fractional crystallization gave the pure compound m.p. 169° mixed m.p. 168·5–169° with authenic acetaldehyde 2:4-dinitrophenylhydrazone (m.p. 169°).

The solution of non-volatile compounds was filtered from a small quantity of oily polymer, an equal volume of saturated brine added, and the

solution repeatedly extracted with ether, and dried with Na_2SO_4, yielding 1·53 g. of material. This was suspended in hot Na_2CO_3 solution and treated with 193 ml. $KMnO_4$ ($N/10$) to oxidize aldehyde to carboxyl, on the assumption that 80 % would be glutaric half-aldehyde methyl ester. The solution was acidified (H_2SO_4), extracted repeatedly with ether, and the extract washed, dried (Na_2SO_4) and evaporated. The residue was first titrated with, and then quantitatively saponified with, alcoholic potash. (Found: COOMe, 14; COOH, 42 %. Calc. for $HOOC.CH_2.CH_2.CH_2.COOMe$: COOMe, 40; COOH, 31 %.) The saponified solution, after dilution with water and evaporation of the alcohol, was acidified (H_2SO_4), saturated with salt, and extracted with ether, first by shaking and then for 8 hr. in a continuous extractor. The former extract gave a brown solid which, after crystallization from chloroform-light petroleum, was dissolved in hot water, filtered and evaporated; it then had m.p. 61–65·5° but could not be identified. The second extract gave a white solid which was similarly treated, then repeatedly recrystallized from benzene, and which yielded a small quantity of glutaric acid, m.p. 92·5–93·5° and mixed m.p. 95·5° with authentic glutaric acid (m.p. 97·5–98°).

To the distillate, consisting of chloroform and aqueous layers, more chloroform was added and the whole titrated with N NaOH, with shaking, in a separating funnel. The chloroform solution was separated, dried (Na_2SO_4) and the solvent removed, the latter was mechanically shaken with an excess of a solution of 2:4-dinitrophenylhydrazine in 2N HCl, when it gave a derivative, which after repeated crystallization, had m.p. 152–152·5° (0·101 g.) and was probably impure acetaldehyde 2:4-dinitrophenylhydrazone. The neutral residue, after removal of chloroform, was distilled, giving a small distillate, b.p. 70–130°; this furnished a 2:4-dinitrophenylhydrazone which after recrystallization had m.p. 100–101·5°. The mixed m.p. with authentic caproic aldehyde 2:4-dinitrophenylhydrazone (m.p. 109°) was 103–104°.

The aqueous solution after evaporation and drying to constant weight in a vacuum desiccator, gave 2·732 g. of sodium salt having equivalent 91·3 (from the amount of N NaOH used). (The same procedure for estimating the equivalent of sodium salts was employed a number of times below.) This was dissolved in water (110 ml.), acidified (H_2SO_4) and distilled; the first 26 ml. and the next 75 ml. of distillate were collected. The last was neutralized and yielded a sodium salt (1·378 g., equiv. 83·7), giving a *p*-bromophenacyl derivative which melted for the most part at 81–85° (*p*-bromophenacyl acetate has m.p. 87–87·5°), but which contained a higher melting impurity. We made a number of attempts but were unable to separate the latter. A portion of the sodium salt was refluxed with dilute H_2SO_4 and the solution distilled; the distillate on neutralization gave a sodium salt having equiv. 83·2 (sodium acetate has equiv. 82), but again we were unable to prepare a pure *p*-bromophenacyl ester.

The first fraction of distillate contained a layer of oily acid which was pipetted off, and the solution again distilled; 6·3 ml. of this distillate were taken, combined with the oily acid and neutralized giving 0·403 g. of a sodium salt, equiv. 127. It was converted into its *p*-bromophenacyl derivative which, after three recrystallizations, had m.p. 70·5–71·5° and mixed m.p. 71–71·5° with authentic *p*-bromophenacyl caproate of m.p. 71·5–72°.

Oxidation of methyl arachidonate by acetone-$KMnO_4$. Methyl arachidonate (5·0 g.) was dissolved in dry acetone (300 ml.) and heated under reflux in an apparatus in which the returning acetone percolated through $KMnO_4$. In the course of 37 hr. 36·6 g. of the last were used without the appearance of a pink coloration; since only 31·4 g. would be necessary to convert each ethylenic link into two carboxyl groups, the oxidation was stopped. The solution was filtered from the manganese mud and the latter washed with acetone. The acetone was evaporated and the residue taken up in light petroleum and washed with NaOH solution and with water (added to bulk aqueous extract below). After drying (Na_2SO_4) and removal of solvent, unchanged ester (1·0 g.) remained.

The manganese mud was dried and leached out with 500 ml. boiling water. The latter after filtration, evaporation and desiccation yielded 11·9 g. of salts (A). The desiccated manganese mud now weighed 19·6 g. The CO_2 content of these two fractions (which contained volatile organic acids) was determined as follows. An aqueous solution of the material was heated to boiling under reflux in a current of CO_2-free air, the top of the condenser being connected to a wash bottle containing $N/10$ $Ba(OH)_2$. Dilute H_2SO_4 was run into the solution and the liberated CO_2 swept through. The baryta solution was filtered and titrated against $N/10$ HCl. The difference from a blank gave the CO_2 trapped, and a correction for imperfections in this method was applied from control experiments with known quantities of carbonate. The extracted, dry, manganese mud and the salts (A) contained 48·9 and 119·4 mg. CO_2/g. respectively.

The salts (A) (3·7 g.) were dissolved in water, acidified (H_2SO_4) and steam distilled.

Successive fractions of distillate (ml.)	10	10	10	150	180
Na salt: wt. (mg.)	123	74	55	321	87
equiv.	103·5	98	97	90	172

A repetition with 7·65 g. of salts (A) yielded three fractions of sodium salts: 0·685 g., equiv. 93; 0·211 g.,

equiv. 86; 0·265 g., equiv. 115. The first of these fractions was dissolved in a minimum of water, acidified (H_2SO_4), extracted repeatedly with ether, and the extract dried (Na_2SO_4) and evaporated. The residue was fractionated by distillation through a series of three bulbs which were successively heated in an air-bath. The fraction (20 mg.) taken at air-bath temperature, 195–225°, was converted via the acid chloride to the anilide which had m.p. 79·5–81·0°, mixed m.p. 80–81·5° with caproic anilide (m.p. 96°), and mixed m.p. 55–56·5° with *n*-valeric anilide (m.p. 61·5–62°). The fraction was thus probably caproic acid. The mid-fraction of sodium salts (0·308 g.) yielded a *p*-bromophenacyl derivative (0·721 g.) of m.p. 70°; five recrystallizations gave *p*-bromophenacyl acetate, m.p. 83–83·5° and mixed m.p. 86° with the authentic compound (m.p. 87–87·5°).

There remained in the steam distillation flask a tar and an aqueous solution; the former (insoluble in benzene and in ether) was dissolved in acetone, dried (Na_2SO_4), and after removal of solvent weighed 0·404 g. The aqueous solution was extracted with ether in a continuous extractor for 22 hr. and with fresh solvent for a further 6 hr. On removal of the ether the residue was dissolved in hot acetone (in which nearly all of it dissolved), filtered, and cooled, when crystals, 49 mg., m.p. 55°, were deposited. After two recrystallizations from benzene-acetone, it had m.p. 57–58°. (Found: C, 84·6; H, 15·0 %. *n*-$C_{27}H_{56}$ has m.p. 58·5–59·5°, C, 85·2; H, 14·9 %.) Addition of two volumes of light petroleum to the acetone filtrate yielded an oil which crystallized. The product after draining and washing with ether had m.p. 99–100° and was dissolved in water, filtered, evaporated and desiccated over H_2SO_4. The residue (45·5 mg.) had m.p. 160–161·5° (decomp.); a portion was converted into the ammonium salt which yielded pyrrole on ignition with zinc dust, indicating succinic acid. A further portion was titrated with NaOH, giving equiv. 51·3, and the solution was acidified (acetic acid), treated with $CaCl_2$ solution, and the precipitate titrated in acid against $KMnO_4$ at 60°, whence 33·2 mg. (anhydrous) oxalic acid were obtained.

The acetone-light petroleum solution on evaporation yielded a residue (C_6H_6), which after washing had m.p. 99–100°. It was dissolved in water, filtered and evaporated and yielded hydrated oxalic acid (41 mg., equiv. 63·2).

Ozonolysis of ethyl linoleate. Corn (maize) oil acids (177 g.; i.v. Hübl = 114) were brominated (40 ml. bromine) in light petroleum solution. The solid product was recrystallized from hot benzene yielding tetrabromostearic acid m.p. 114–115°. It (44 g.) was reduced with zinc dust according to Brown & Fraenkel [1938]. The linoleic acid was esterified with EtOH-HCl and gave ethyl linoleate (12·8 g.), b.p. 173·5°/0·5 mm.; i.v. Hübl = 161 (calc. 164·8); this (3·5 g.) was ozonized as before, saturation being reached after 9 hr. The chloroform was removed as far as possible in a water pump vacuum at 40°, yielding a colourless oil (4·90 g. Theory, assuming a *bis*-ozonide-peroxide, 4·75 g.).

Cold water (35 ml.) was added to the ozonide and decomposition was allowed to proceed in apparatus similar to that used for methyl arachidonate except that five small wash bottles were employed, the first containing a saturated solution of 2:4-dinitrophenylhydrazine in 2*N* HCl, the second the same reagent in alcoholic H_2SO_4 and the last three *N* NaOH (75 ml. each). The water-bath was raised to boiling point over a period of 1 hr. and a slow current of CO_2-free air continued overnight. The contents of the 2:4-dinitrophenylhydrazine wash bottles were then replaced by fresh reagent in 2*N* HCl solution, and steam passed in place of the air current, aspiration through the wash train being continued. Finally the distillate and the solution of non-volatile material in the flask were both saturated with salt and extracted with ether and the latter dried (Na_2SO_4).

The NaOH solution was cooled and titrated with *N* HCl, using phenolphthalein and methyl orange successively and 0·259 g. CO_2 was found.

Of the 2:4-dinitrophenylhydrazine solutions, the alcoholic one gave no appreciable yield, whilst the first and second aqueous ones gave respectively two products (0·151 and 0·035 g.), m.p. 107–109° and 87°, which on recrystallization had m.p. 145·5–147·5° and 137–143°. These were combined and after four recrystallizations had m.p. 160° and mixed m.p. 168–169° with authentic acetaldehyde 2:4-dinitrophenylhydrazone (m.p. 168·5–169°).

Evaporation of the ethereal solution of non-volatile products gave a semi-solid mass which was washed with ether-light petroleum yielding crude azelaic acid (0·122 g.), m.p. 100·5–102·5°. The residue from evaporation of the washings was suspended in hot Na_2CO_3 solution and titrated with $KMnO_4$ until the reaction slowed down (required 63·9 ml. of $KMnO_4$, 0·06*N*). The solution was acidified (H_2SO_4), extracted with ether, the extract washed, dried (Na_2SO_4), and evaporated, giving a semi-solid (1·286 g.). This was dissolved in alcohol and titrated with alcoholic potash and then saponified with excess of this reagent. (Found: carboxyl equiv. 213 and ester equiv. 216; $HOOC.(CH_2)_7COOEt$ has mol. wt. 205). The solution was acidified (HCl), cooled and filtered, yielding crude azelaic acid (0·641), m.p. 102·5–103°. The azelaic acid was recrystallized once from hot water, when it had m.p. 107·5–108° alone, or mixed with authentic azelaic acid (m.p. 106·5–108°).

The ethereal solution of the distillate was titrated, with shaking, in a separating funnel with *N* NaOH. No pure product was isolated from the neutral ether; a carbonyl group was present since reaction occurred with 2:4-dinitrophenylhydrazine but no identifiable hydrazone was isolated; a semicarbazone could not be formed.

The aqueous solution gave a sodium salt (0·875 g.), equiv. 135. This was dissolved in water (55 ml.), acidified (H_2SO_4) and distilled. The first 20 ml. and the next 29 ml. of distillate gave Na salts, 0·380 g., equiv. 136, and 0·317 g., equiv. 132, respectively. The former was converted into its *p*-bromophenacyl derivative which had m.p. 71·5–72° and mixed m.p. 71–72° with authentic *p*-bromophenacyl caproate (m.p. 71·5–72°).

SUMMARY

1. The results of ozonolysis and of acetone-$KMnO_4$ oxidation of methyl arachidonate are in agreement with the constitution (II) $\Delta^{5.8.11.14}$-eicosatetrenoic acid, for arachidonic acid, which was proposed by Dolby *et al.* [1940].

2. Contrary to the results of Takahashi [1921] ozonolysis of ethyl linoleate yields products in accordance with the generally accepted structure for linoleic acid, $\Delta^{9.12}$-octadecadienoic acid (IV).

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Studies on Diffusing Factors

10. NOTE ON THE FORMATION OF VISCOUS MATERIALS BY *CLOSTRIDIUM BUTYLICUM*

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The enzyme system known as hyaluronidase, which exists in some tissues and bacteria, hydrolyses polysaccharides of the hyaluronic acid type. Hyaluronic acid is built up from equimolecular amounts of acetylglucosamine and uronic acid residues [Meyer, 1938]. In the hyaluronidase complex two well-defined individual components have so far been detected [East, Madinaveitia & Todd, 1941]. One of them reduces the viscosity of the viscous polysaccharide without liberating any appreciable amount of reducing sugar. The other is a glucosaminidase which does not attack the viscous polysaccharide but which may play a role in the later stages of the hydrolysis of the polysaccharide by splitting glucosaminic linkages. It is possible that a third component also exists, a uronidase which would hydrolyse the uronidic linkages of the polysaccharide. No evidence has so far been obtained for the presence of such an enzyme in the preparations assayed. The substrate used was menthol glucuronide.

The presence of diffusing factors has been demonstrated in all the sources of hyaluronidase as yet known (e.g. in mammalian testicle, leech extract, snake venoms and some bacteria). It has been claimed that hyaluronidase and diffusing factor are the same substance [Chain & Duthie, 1940; McClean, 1941*b*; McClean & Hale, 1941]. There is, however, a discrepancy between the viscosity-reducing power